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Hypoderma rubi on two new hosts in Slovakia

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Abstract—*Hypoderma rubi* was found on the previous year's fallen petioles of *Fraxinus chinensis* subsp. *rhynchophylla* and on attached dead twigs of *Rhododendron fortunei* in Slovakia. The fungus, which is recorded for the first time on these host taxa, also represents a new taxon for the Slovak mycota. The morphological characteristics of the fungus found on Slovak collections are described.

Key words—Chinese ash, Fortune's rhododendron, morphology, *Rhytismataceae, Rhytismatales*

Introduction

Hypoderma rubi, the type and best-known species of *Hypoderma* (*Rhytismataceae*, *Rhytismatales*), occurs on a large number of herbaceous and woody plant genera and has an extensive geographical distribution. According to Farr & Rossman (2017), most records are from Asia (China, India), although the fungus has also been recorded in New Zealand, North America (USA), Central America (Panama), and Europe (Czech Republic, Germany, Spain, Sweden, Ukraine, United Kingdom). European findings of the fungus are mainly on the type host genus *Rubus* L. (Hilitzer 1929, Lotz-Winter et al. 2011, Moreno et al. 2004, Eriksson 2014, Lantz et al. 2011, Dudka et al. 2004, Cannon et al. 1985). There are also some specimens of *H. rubi* from other European countries (Channel Islands, Italy, Norway), Asia (Japan), South America (Chile), and Africa (Malawi) in the K & IMI

fungaria (Royal Botanic Gardens, Kew) that have not yet been formally published.

In 2015, during a survey of the mycota of trees and shrubs in Mlyňany Arboretum of the Slovak Academy of Sciences, two collections of *H. rubi* were made. This species is not listed in the checklist of fungi of Slovakia (Lizoň & Bacigálová 1998), and there are no previous records of it in Slovakia. Petrak's records of *H. rubi* on *Rubus* spp. (deposited in international herbaria, e.g. BPI, IMI, K) collected in the former Czechoslovakia, originate in the territory of Czech Republic. This paper presents, therefore, the first collections of *H. rubi* from Slovakia, and the first on *Fraxinus chinensis* subsp. *rhynchophylla* (Hance) A.E. Murray and *Rhododendron fortunei* Lindl. A description and illustrations of the fungus based on the Slovak collections are provided.

Materials & methods

The previous year's petioles of *F. chinensis* subsp. *rhynchophylla* and attached dead twigs of *R. fortunei* were collected in the Mlyňany Arboretum (south-west of Slovakia) in autumn 2015. The specimens were examined using an Olympus SZ61 stereomicroscope and Olympus BX51 standard light microscope. Morphological and microscopic examinations were carried out on dried material rehydrated in water. Lactophenol blue solution (Merck, Darmstadt) was used to stain hyaline structures. The morphological structures of the fungus were photographed using an Olympus SP350 digital camera. We compared our measurements with previously published descriptions. Voucher specimens are deposited in the Plant Pathology Herbarium of the Institute of Forest Ecology of Slovak Academy of Sciences, Nitra, Slovakia (NR).

Taxonomy

Hypoderma rubi (Pers.) DC. ex Chevall.,
J. Phys. Chim. Hist. Nat. Arts. 94: 31 (1822) Figure 1

= *Hypoderma virgultorum* DC., Fl. Franç., Ed. 3, 6: 165 (1815), nom. illeg.
= *Hypoderma commune* (Fr.) Duby, Mém. Soc. Phys. Hist. Nat. Genève 16: 53 (1861)

Hysterothecia embedded in the previous year's fallen Chinese ash petioles and in paler portions of Fortune's rhododendron twigs, abundant, subcuticular, black, elliptical to elongated-elliptical, raising the substrate surface, (0.4–)0.8–2.7 × 0.3–0.6 mm, opening by a single longitudinal split, lips grey, clypeus thickened towards the lips. Lip cells hyaline, 14–20.5 × 2.5–3.5 µm. Asci unitunicate, clavate, long-stalked, (75–)90–125 × 10–14.5 µm, 8-spored. Ascospores hyaline, aseptate, guttulate, elongated-

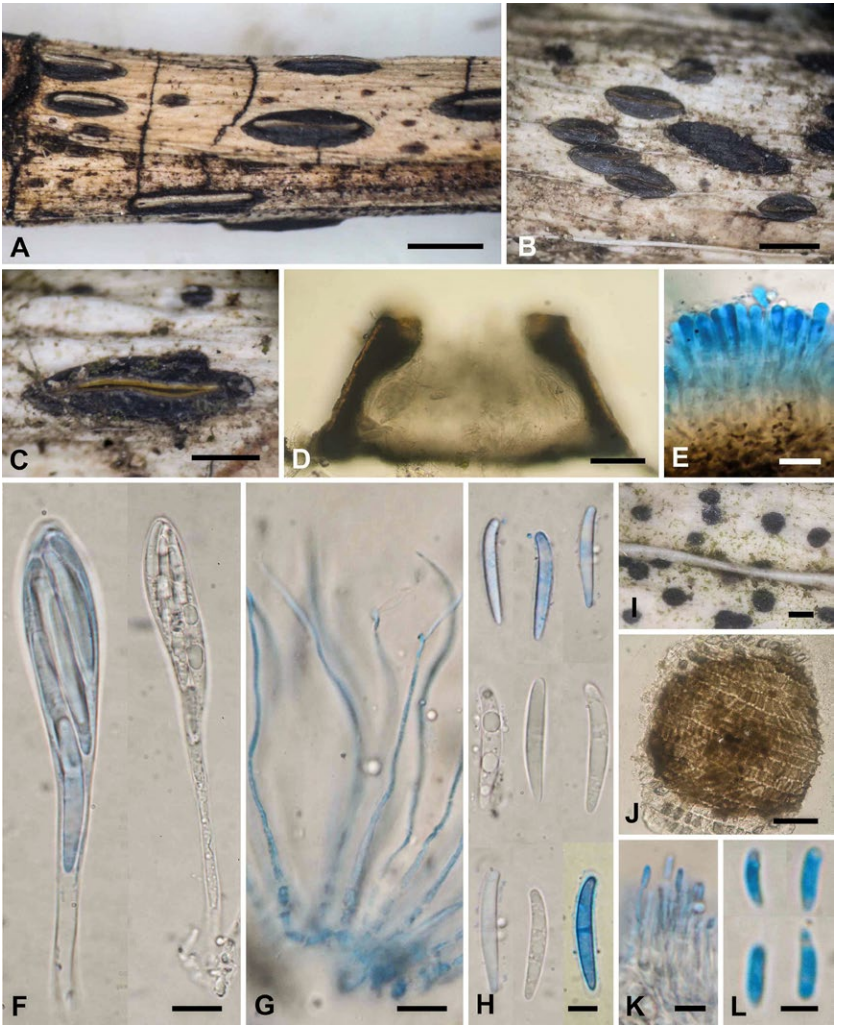

Figure 1. *Hypoderma rubi* (A, C–H = NR 5477; B, I–L = NR 5478). A: Hysterothecia, pycnidia and zone lines on *Fraxinus* petiole; B: Hysterothecia on *Rhododendron* twig; C: Mature hysterothecium (surface view); D: Hysterothecium in vertical section; E: Lip cells; F: Asci with ascospores; G: Paraphyses; H: Ascospores; I: Pycnidia (surface view); J: Pycnidium (detail of surface appearance); K: Conidiogenous cells with conidia; L: Conidia. Scale bars: A, B = 1 mm; C = 0.5 mm; D = 100 µm; E, H, K = 5 µm; F, G = 10 µm; I = 200 µm; J = 50 µm; L = 2 µm.

clavate to cylindrical, bent towards the top end, 18–25 × 2.5–3.5 µm, usually not covered by a gelatinous sheath but sometimes clad in a thin sheath.

Table 1. Biometric characteristics of *Hypoderma rubi* from the Slovak material and as reported by other authors.

Morphology	Cannon & Minter (1986)	Johnston (1990)	Slovak material
Hysterothecia (mm)	1.4–2.5(–3.0) × 0.5–0.75	0.8–3.0 × 0.3–0.6	(0.4–)0.8–2.7 × 0.3–0.6
Asci (µm)	87–130 × 12–14.5	110–160 × 11–14	(75–)90–125 × 10–14.5
Ascospores (µm)	(16–)20–24(–30) × 2.5–3.5	15–28 × 2.5–5.5	18–25 × 2.5–3.5
Pycnidia (µm diam.)	75–250(–300)	100–300	(80–)105–220
Conidia (µm)	2–4 × 0.75–1	3–5 × 1–1.5	2–4 × 1

Paraphyses hyaline, filiform, septate, branched or unbranched, not swollen at the apex, as long as mature asci, covered by a gelatinous sheath. Pycnidia subcuticular, black, circular, (80–)105–220 µm diam., opening by 1–2 small central ostioles, accompanying the hysterothecia. Conidiogenous cells cylindrical, tapering towards the apex, hyaline, 7–12 × 1–2 µm. Conidia cylindrical, sometimes allantoid, hyaline, aseptate, 2–4 × 1 µm. Zone lines black, thin.

Specimens examined—**SLOVAKIA. Vieska nad Žitavou, Mlyňany Arboretum,** 48.3203°N 18.3668°E, on the previous year's petioles of *Fraxinus chinensis* subsp. *rhynchophylla* (*Oleaceae*), 2 Oct. 2015, leg. et det. K. Pastirčáková (NR 5477); 48.3208°N 18.3692°E, on dead attached twigs of *Rhododendron fortunei* (*Ericaceae*), 9 Nov. 2015, leg. et det. M. Pastirčák (NR 5478).

The fungus was identified as *Hypoderma rubi* based on morphological description and measurements of reproductive structures. A comparison of some biometric characteristics of *H. rubi* on *Rubus* sp. (Cannon & Minter 1986), on different host plants (Johnston 1990), and of the Slovak material is given in Table 1. Morphometric data of the Slovak collections correspond well to previously published descriptions. Although ascospores usually are not covered by a gelatinous sheath, a thin sheath was often observed after staining (Fig. 1H). Hou et al. (2007) also recorded *H. rubi* on *Cunninghamia lanceolata* (Lamb.) Hook. (*Cupressaceae*) with ascospores clad in a thin gelatinous sheath.

The findings of *H. rubi* on *F. chinensis* subsp. *rhynchophylla* and *R. fortunei* from Slovakia represent the first records of the fungus in the country and new host taxa for the fungus.

Discussion

Rubus is the principal host genus on which *Hypoderma rubi* is most commonly collected (Minter 1984). However, according to Farr & Rossman

(2017), *H. rubi* has been recorded on leaves, stems, and petioles of species representing 35 host genera in 27 families. The species therefore seems to be widely plurivorous, although Cannon & Minter (1986), who doubt the conspecificity of the fungi on these hosts, suggest that numerous taxa may be involved. According to Lantieri et al. (2011), the genetic relationships amongst specimens identified as *H. rubi* are poorly resolved and the application of this name by different authors is uncertain. There are as yet very few molecular data available for *H. rubi* and from only a few hosts (Lantieri et al. 2011, Lantz et al. 2011). A comprehensive phylogenetic study is necessary to determine their identity and relationships.

Hypoderma rubi on *Rhododendron* sp. has been recorded in Europe (Wales; Smith 1951) and Asia (Malaysia; Spooner 1991). In China, four other *Hypoderma* species are known on *Rhododendron* spp.: *H. rhododendri-mariesii* Y.R. Lin & S.J. Wang on *R. mariesii* Hemsl. & E.H. Wilson (Lin et al. 2004), *H. cuspidatum* C.L. Hou & M. Piepenbr., *H. shiqii* C.L. Hou & M. Piepenbr., and *H. urniforme* C.L. Hou & M. Piepenbr. on *Rhododendron* sp. (Hou & Piepenbring 2006, Hou et al. 2007). They differ from *H. rubi* mainly by the shape and position of their hysterothecia as well as ascospore characteristics. *Hypoderma rhododendri-mariesii* has smaller (<860 µm long) hysterothecia, asci with cylindric-fusiform ascospores, and shorter ((9–)11–17 µm) ascospores; *H. cuspidatum* has intraepidermal hysterothecia with acute ends, ascospores with thick gelatinous sheaths, and unbranched paraphyses; *H. shiqii* has intraepidermal hysterothecia without lips that often open with one slit and additional lateral fissures, clavate asci with bifusiform ascospores, and ascospores with thick gelatinous sheaths; and *H. urniforme* is characterised by urniform, intraepidermal hysterothecia with red-brown lips and almost cylindrical asci with ellipsoidal to long-ellipsoidal ascospores with irregular gelatinous sheaths. *H. cuspidatum, H. shiqii,* and *H. urniforme* often occur together on the same twigs (Hou et al. 2007).

On-line databases of the British Mycological Society (Fungal Records Database of Britain and Ireland, http://www.fieldmycology.net) and Royal Botanic Gardens, Kew (Herb. IMI database, http://www.herbimi.info) include data on fungarium specimens of *H. rubi* on dead fallen leaves and dead attached twigs of *Rhododendron ponticum* L., *R. sinogrande* Balf. f. & W.W. Sm. and *R.* sp. from Scotland (IMI 358124b, 358144, 358155, 358157, 358158a); and on dead fallen petioles of *Fraxinus excelsior* L. from England (IMI 339025, K(M)160355). These records are evidently not yet published.

In the USA, Hanlin (1963) recorded *Hypoderma commune* on *Fraxinus americana* L.; however Powell (1974) treated *H. commune* as a synonym of *H. rubi*. Based on the available literature and electronic resources, we consider that only one *Hypoderma* species, *H. rubi,* has been recorded on *Fraxinus*.

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